IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	*Application Number*	09/724,265
	Filing Date	Nov. 28, 2000
	First Named Inventor	Bruce Marvin HELD et al.
	Group Art Unit	1638
	Examiner Name	G. Helmer
	Attorney Docket Number	N1205-008
Title of the Invention: **Methods and Compositions for the Introduction of Molecules Into Cells**		

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P O Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Herbert Martin Wilson, of 1915 Stevenson Drive, Ames, Iowa 50010, hereby declare that:

I graduated from University of Leicester (United Kingdom) in 1975 with a Ph.D. in Plant Cell Biology.

I was employed by Pfizer, Inc., from 1982 to 1986, where I was a senior scientist in the Plant Genetics Department.

I was employed by ICI Seeds, Inc., from 1986 through 1994 where I was Cell Biology Project Leader.

Since 1995, I have been employed by Stine Seed Company as Director of Stine Biotechnology.

I am one of the co-inventors of the invention described and claimed in the above-identified application and am familiar with the Office Action dated January 30, 2003.

The Examiner states in his considerations of our claims 1, 2, 4, 7-10, 13-20, 56, 57, 61 and 63 that Mets in U.S. Patent 5,240,842 is silent on flow rate. The present invention has claimed flow rates of between 1 and 350 ul/minute (Claim 1 (b), page 56) with the most preferred rate being between 8.0 and 50.0 ul/minute (Claims 58, 61, 59 and 63). I have described below the rates used by Mets, the results as they relate to

flow rate, and why the present invention represents an unexpected improvement in the application of the aerosol beam injector to delivery of molecules to cells.

In the Examples cited in the Mets '842 patent the nebulizer used is described as model 4207 from Inhalation Plastic Inc. (Column 9, lines 40, 41 and 42). For a picture of the nebulizer see Attachment 1. This nebulizer is of the Lovelace design. Professor Otto Raabe reports (see Attachment 2) that this nebulizer operates at a flow rate of around 35 ul/minute per liter of carrier gas. In the examples in the Mets patent a carrier gas flow rate of 4 liters/minute and a pressure of 30 psi was used in all cases. This translates to a sample flow rate of 140 ul/minute. Under these conditions droplets at the point of impacting target cells were around 2 microns in diameter (see below).

Mets describes transformation of Chlamydomonas cells using the aerosol beam injector operating using a nebulizer of the Lovelace type as described above. As a target population, Mets used 10^6 cells in 100 ul of medium (this figure was obtained from the file wrapper since there is an obvious misprint in the patent itself where target population is given as 10 cells/ml - clearly an impossibility). From this population, Mets reports obtaining two transformants after beaming (Example 4). Survival following beaming was simply reported as greater than 0.1% of the impacted cells (column 11, lines 15-17). Droplets generated in the nebulizer of the type used by Mets are large. At the point of impacting cells (after passing through tubing and the nozzle (Figs 1, 2 and 3 of Mets patent) they are described as being 2 microns in diameter (0.1 microns in Example 2 - but here no delivery of molecules to cells was noted). Although droplet size at point of impact was not measured in the current application it is clear that droplet size must be appreciably less than 2 microns since efficient transformation of bacterial cells is reported, E. Coli cells that are on average 1.5 microns in length and 0.75 microns in width.

In our application we show that the most effective delivery of molecules to cells occurs at flow rates of between 4.0 and 50.0 ul/minute (page 50, Table 6 of the application). Higher flow rates are not as useful and result in significant tissue and cell damage. This is a surprising result and is not anticipated by Mets.

Microflow nebulizers have been used mainly for ICP (inductively coupled plasma-atomic emission spectrometry). These nebulizers generate significant shearing forces that result in production of small, uniformly sized droplets. The large number of small droplets allows for efficient analysis of samples. Nebulizers of this type, however, are expected to break up large macromolecules such as DNA. Surprisingly, in the present invention, this was found not to be the case because in our application of the present invention we demonstrate the introduction of DNA that is capable of expression in bacteria and plant cells (example 2 page 28; example 3 page 31; example 4 page 32; example 5 page 33; example 6 page 35; example 7 page 35; example 10 page 41; example 11 page 42; example 12 page 44; and examples 17-19 page 49). Surprisingly, in the present invention, nebulizers of this type are the preferred type for use for delivery of macromolecules such as DNA into cells.

In summary, unexpectedly, delivery of small droplets at low flow rates is essential for the successful use of the aerosol beam injector as a means of DNA delivery to cells in the present invention. This can best be achieved by use of a microflow nebulizer in the aerosol beam injector.

Despite great interest in alternative methods of introducing DNA into living cells, all previous methods of using aerosol beam injection have not been successful. Mets used flow rates that are not optimal and in fact result in extremely low or negligible rates of transformation. Use of low flow rate as described in the current application now makes aerosol beam injection a reproducible and efficient method.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Herbert Martin Wilson 7/17/03

Herbert Martin Wilson, Ph.D.